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Some Effects of Chemotherapeutic Drugs on Bone Marrow Stem Cells

II. Effect of non-Hodgkin Lymphoma Chemotherapy on Various Hemopoietic Compartments of the Mouse

Dominique Duménil, Françoise Sainteny, and Emilia Frindel

Institut de Radiobiologie Clinique (U66 INSERM) and Laboratoire Associé N° 207 CNRS, 16 bis, Avenue Paul Vaillant-Couturier, F-94800 Villejuif, France

Summary. *The non-Hodgkin lymphoma chemotherapy protocol used at the Gustave-Roussy Institute was adapted, in terms of drug doses and interval between doses, to normal CBA mice. The numbers of pluripotential stem cells (CFU-S), unipotential stem cells (CFC), differentiated bone marrow cells, and circulating white cells were determined.*

Eight hours after each drug of the first chemotherapy cycle the number of pluripotent stem cells decreased while the proportion of these cells in DNA synthesis increased.

Six hours after the end of each complete cycle, the stem cell compartments were found to be considerably depleted, and they were not completely restored when the next cycle was begun, while the other hematologic compartments were completely restored at this time.

Introduction

The utilization of chemotherapeutic drugs is limited, because of their toxicity, to normal tissues, particularly with respect to the hemopoietic tissues. In patients only mature cells and the unipotential bone marrow cells committed to differentiation towards the formation of a particular cell line can be monitored. It is therefore useful to try and adapt the chemotherapy protocols to a mouse model in order to study the effects of drugs on pluripotent stem cells and their consequences on hemopoietic lineages.

Many research groups have studied the toxicity of drugs on the bone marrow [3, 6, 2, 10, 5, 13]. Rarely, however, has a complete clinical chemotherapeutic regimen been applied and tested with animal models. Jenkins et al. [9], for example, have studied the time course changes in the number of CFU-S of normal rats 24 h after the last drug injection utilized in Hodgkin's disease, but these authors did not correlate their results with observations in the other hematologic compartments.

In this work, we studied simultaneously the CFU-S, CFC, and more mature compartments in the same mouse. We adapted to normal mice the chemotherapeutic protocol utilized for human subjects with non-Hodgkin lymphoma, and followed its effects during the first cycle of chemotherapy and 6 h and 12 days after the end of each chemotherapy cycle.

Materials and Methods

Mice

Experiments were performed on CBA/OLA male and female SPF mice aged 2 months, weighing an average of 20 g. Each treated group consisted of 50 mice. Each irradiated recipient group consisted of 10 mice. In all, 435 mice were used for these experiments.

Protocol

The chemotherapeutic protocol employed at the Gustave-Roussy Institute for the treatment of non-Hodgkin lymphoma is as follows:

Day 1: Adriamycin (40 mg/m^2 IV),

Day 2: VM26 (50 mg/m^2 IV), (*N*-Demethyl-epipodophyllotoxine thenylidene glucoside),

Days 3, 4, 5: Cyclophosphamide (300 mg/m^2 IV),

Days 4–7: Prednisone (30 mg/m^2 PO) or Methylprednisolone (30 mg IV).

This series is given once a month for 6 months.

There were two problems in the adaptation of the above protocol to mice: the drug doses and the time intervals. As suggested in the literature [7], we used 0.3% of the dose used in patients. The drugs were injected IP because of technical difficulties of repeated IV injections in mice. Since the cell cycle duration of mouse bone marrow is about one-third that of man [8], we reduced the time intervals used in patients to one-third, i.e., every 8 h instead of every 24 h.

Reprint requests should be addressed to: Dr. E. Frindel at Institut Gustave-Roussy, 16 bis, Avenue Paul Vaillant-Couturier, 94800 Villejuif, France

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The protocol for each treatment cycle was as follows:
Day 1 a 8.00 a.m.: Adriamycin (0.125 mg per mouse IP),
Day 1 at 4.00 p.m.: VM26 (0.15 mg IP),

Day 1 at 12.00 midnight Day 2 at 8.00 a.m. Day 2 at 4.00 p.m.	}	Cyclophosphamide (0.9 mg IP)
Day 2 at 8.00 a.m. Day 2 at 4.00 p.m. Day 2 at 12.00 midnight Day 3 at 8.00 a.m.	}	Methylprednisolone (0.09 mg IP)

A total of five cycles was administered, with an interval of 12 days between cycles.

The following parameters were tested 8 h after the injection of each drug during the first chemotherapy cycle and 6 h and 12 days after the end of each chemotherapy cycle: the number of CFU-S and their percentage in DNA synthesis (S phase), the number of CFC, the number of nucleated bone marrow cells, and the number of leukocytes in the blood.

The number of CFU-S was determined by the technique of Till and McCulloch [12]: an appropriate dilution of the bone marrow nucleated cells was made for the injection of 0.2 ml IV to mice previously given 900 rads of whole-body γ-irradiation (Cesium). Nine days later the mice were killed and their spleens were removed and fixed in Bouin's solution. Macroscopic colonies on the spleens were scored 24 h after fixation.

The percentage of CFU-S in S-phase was determined by the suicide method of Becker et al. [1] with the aid of high-specific-activity ^{3}H-TdR.

The number of CFC was studied by the technique of Worton et al. [14]. We cultured 10^5 bone marrow cells in α medium containing serum, methyl cellulose, and colony-stimulating factors (CSF). Colonies were counted 7 days after incubation at 37° C.

Results

The results represent the average of two or three separate experiments.

CFU-S and CFC

All the results are expressed per leg. Results per 10^5 are given in Table 1.

1. During the First Chemotherapy Cycle (Fig. 1). Eight hours after the injection of adriamycin the number of CFU-S was the same as in controls. These CFU-S were quiescent. The number of CFC was slightly decreased (73% of the control value).

Eight hours after the VM26 injection (i.e., 16 h after the adriamycin was injected) the number of CFU-S was 60% of the control value, with 16% of these cells in DNA synthesis. The number of CFC was 66% of the control value.

Eight hours after the cyclophosphamide injection (or 16 h after the VM26 and 24 h after the adriamycin injection) there were only 36% of CFU-S remaining, with 34% of these cells in S phase. The number of CFC was 47% of the controls.

Eight hours after the injection of methylprednisolone (16 h after the injection of cyclophosphamide, 24 h after VM26, and 36 h after adriamycin) there was approximately 10% survival of the CFU-S, 23% of which were in S phase. The CFC number was about 11% of the control value.

2. During the Intervals Between the Cycles (Table 2 and Fig. 2). Six hours after the end of the first and the second chemotherapy cycles the numbers of CFU-S and CFC were reduced to 5% and 10%, respectively, of the control values.

Table 1. Survival of CFU-S and CFC expressed as percent of controls

	CFU-S		CFC	
	per 10^5 as % of controls	per leg as % of controls	per 10^5 as % of controls	per leg as % of controls
During first chemotherapy cycle				
8 h after adriamycin	98	98	73.5	73.5
8 h after VM26	72	60	79	66
8 h after cyclophosphamide	52	36	68	47
8 h after Solu-Medrol	32	10.5	34	11
During intervals between cycles				
6 h after 1st cycle	31	9	62	17
12 days after 1st cycle	54	60	44	48
6 h after 2nd cycle	53	8	41	6
12 d after 2nd cycle	65	55	66	55
6 h after 3rd cycle	43	12	82	23
12 d after 3rd cycle	71	92	38	49
6 h after 4th cycle	120	43	160	59
12 d after 4th cycle	62	49	77	59
6 h after 5th cycle	36	13	21	7
20 days after 5th cycle	147	111	170	133

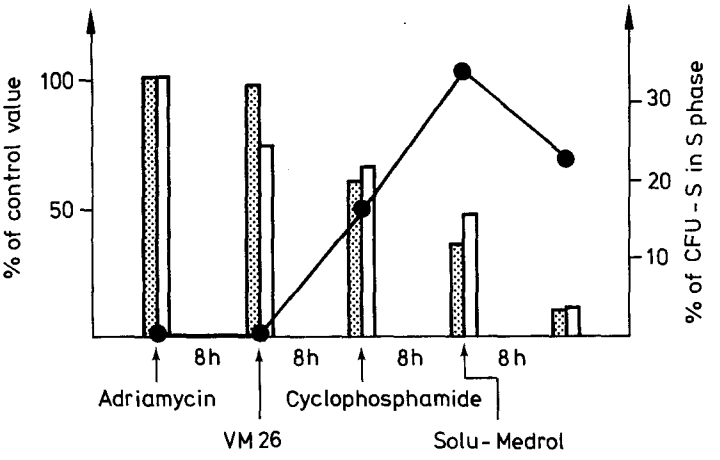

Fig. 1. Time course changes in the number of CFU-S (▦) and CFC (□) per leg, expressed as percentages of control values, and percentage of CFU-S in DNA synthesis (–) 8 h after each drug injection

Twelve days after each of the first two chemotherapy cycles, the number of CFU-S and CFC had recovered to 60% of the control values.

Six hours after the end of the third chemotherapy cycle, the number of CFU-S was 17% of the control value, and the number of CFC 36% of the control value. Twelve days later the number of CFU-S was equal to that in the controls, while the number of CFC remained at 60% of the control value.

Six hours after the fourth cycle the numbers of CFU-S and CFC were 27% and 44% of those in controls, respectively. Twelve days later, CFU-S and CFC were 41% and 44% of the control values, respectively.

Six hours after the fifth cycle the number of CFU-S was 11% of the control value and the number of CFC was 6%.

Twenty days after the end of the treatment the numbers of CFU-S and CFC were no different from those in the controls.

White Blood Cells

Six hours after each chemotherapy cycle the number of WBC was observed to be 4,000 or 5,000/mm³, while the control average was 10,000/mm³. Total recovery of the control level was observed 12 days after each cycle (Table 1).

Table 2. Number of CFU-S, CFC, and nucleated cells per leg and WBC/mm³ during the treatment

	CFU-S per leg	CFC per leg	Nucleated cells per leg $\times 10^6$	WBC/mm³
Controls	3,516 ± 216[a]	12,189 ± 853	15.9 ± 0.7	10,578 ± 1,156
Time after first chemotherapy cycle				
6 h	319 ± 24.4	2,043 ± 180	2.8 ± 0.45	7,066 ± 1,600
12 days	2,116 ± 17.3	5,868 ± 665	17.4 ± 0.34	12,625 ± 1,800
Time after second chemotherapy cycle				
6 h	277 ± 5.6	753 ± 100	2.45 ± 0.15	4,465 ± 435
12 days	1,942 ± 16	6,784 ± 759	13.4 ± 0.35	11,325 ± 2,400
Time after third chemotherapy cycle				
6 h	432 ± 63	2,835 ± 225	4.5 ± 0.4	3,500 ± 730
12 days	3,228 ± 20	5,945 ± 861	20.5 ± 0.25	12,700 ± 1,930
Time after fourth chemotherapy cycle				
6 h	1,512 ± 5.4	7,168 ± 765	5.6 ± 0.45	–
12 days	1,722 ± 98	7,257 ± 984	12.3 ± 0.5	19,700 ± 1,600
Time after fifth chemotherapy cycle				
6 h	448 ± 44	924 ± 145	5.6 ± 0.3	5,400 ± 945
20 days	3,923 ± 192	16,200 ± 1,320	12 ± 0.5	4,600 ± 855

[a] Standard error of the mean

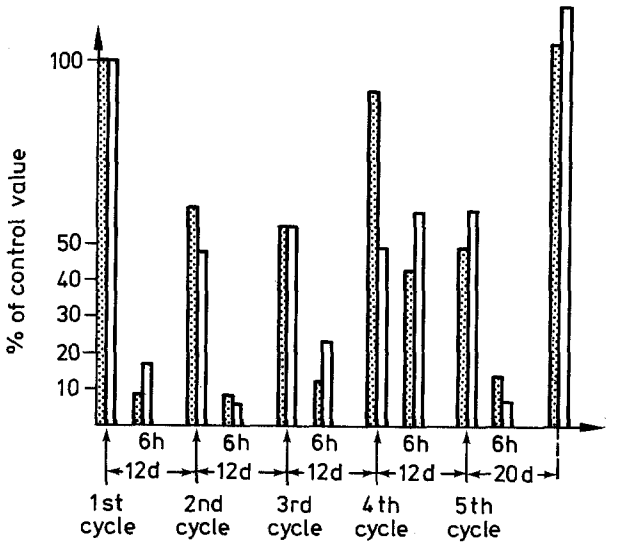

Fig. 2. Time course changes in the number of CFU-S (▦) and CFC (□) per leg, expressed as percentages of control values 6 h and 12 days after the end of each chemotherapy cycle

Discussion

Bone marrow deficiency and leukopenia are frequent consequences of chemotherapy in cancer patients [11]. In clinical cases, only the mature cells and the committed stem cells can be assessed. However, the pluripotent stem cell may be the most important element in the control of homeostasis. We therefore chose a mouse model, since it was possible to study the kinetics of stem cells in this animal.

A total of five cycles of the non-Hodgkin lymphoma chemotherapy protocol was administered to normal mice.

Six hours after the end of each cycle, the numbers of stem cells, nucleated bone marrow cells, and the peripheral WBC decreased. The toxicity of drugs to stem cells appears to depend on the proliferative state of the cells at the time of injection of the drug. At the first injection, the CFU-S were quiescent and not perturbed by the adriamycin administration. Following the first injection, the CFU-S were recruited into cell cycle and became susceptible to the subsequent drug injections.

About 50% of CFC are in DNA synthesis, and therefore more than 50% of these cells are in cycle, which explains their sensitivity to adriamycin, a cycle-dependent drug.

The degree of depletion observed immediately after the end of each cycle does not seem to increase with the number of cycles. This suggests that in the normal mouse, the capacity of restoration remains intact during the course of treatment.

At the start of the next cycle, i.e., 12 days after the end of the previous cycle, the number of cells in the mature compartments returned to normal values, whereas the stem cell compartments were about 50% of the control values. This may be explained by the fact that stem cell differentiation continues during the period between cycles in order to replenish the compartment of functional cells. Twenty days after the fifth and last cycle, the number of cells in all the compartments of the bone marrow was normal.

The difference between the complete recovery in mice and leukopenia in man [11] is difficult to explain. One reason may be that the mice we are dealing with are young, normal, SPF animals, which may not be the precise model for the treatment of a non-Hodgkin lymphoma. It is now known that one of the differences between the normal and tumor-bearing mouse models is that the CFU-S of tumor-bearing mice are in cell cycle [4]. It is very likely that the original kinetic status of the bone marrow is important for consecutive treatments. The leukopenia in man could be considered to be the result of increased killing of stem cells if most of them are in cell cycle. A greater proportion of this population would thus be sensitive to the drug.

The correlation between CFU-S and CFC in normal mice has been shown by Wu et al. [15]. The present study has shown that in perturbed marrow there is also a good correlation (0.898) between CFU-S and CFC. There is no significant correlation between CFU-S and peripheral leukocytes. Therefore it seems that CFC studies in man may provide useful indirect information on the effect on the pluripotent stem cells, whereas WBC counts seem to give little indication of the effect of drugs on the stem cell compartments.

It is now our aim to determine whether the mouse bone marrow that is apparently restored after the protocol used will show late effects, such as resistance or alternatively higher sensitivity, to subsequent drug treatments. We are also interested to see whether this protocol will provoke secondary leukemia.

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